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The Rapid Conversion of Whey to Yeast

Disposal of whey as waste is becoming increasingly costly, yet its utilisation as animal protein will hardly achieve more than save the expense of treating it as waste. The production of yeast protein from whey is more profitable, however, and the process described here, based on the propagation of Saccharomyces fragilis, *may make this method of whey utilisation a commercial proposition. The cost has been reduced by decreasing the time required to produce the maximum yeast yield and by using raw whey and non-sterile conditions of growth. Maximum yeast production occurs in 4 hr.*

Aaron E. Wasserman*, B.S., Ph.D.

THE cheese manufacturer, faced with the problem of disposing of approximately 9 lb. of whey for every pound of cheese manufactured, has recourse to either of two solutions: whey utilisation or waste disposal.

Disposal of whey as a waste, however, is becoming legally more difficult and more costly. Whey is an excellent source of food for micro-organisms, and large quantities of oxygen are needed for the combustion and assimilation of the high lactose content. Unless oxygen is supplied artificially, the natural reservoir of the gas, formed by the oxygen dissolved in the liquid, may be insufficient to supply the needs for sugar dissimilation, and anaerobic decomposition may occur. Porges, in a previous review in this journal[1], discussed an analagous situation for dairy waste in which the oxidation of 100 lb. skim milk solids in the waste required all the oxygen dissolved (8.4 p.p.m. 25° C.) in almost 12 million Imperial gal. of water. Since the chemical oxygen demand of whey is 40 to 50 times greater than that of the average dairy waste, the difficulty of treating whey by natural aeration can be appreciated.

An economic need

The utilisation of whey in a wide variety of foods and as a starting material for a number of fermentations has been discussed by Whittier and Webb[2], and more recently in an excellent review by Wix and Woodbine[3]. However, whey utilisation has not been considered economically promising, and exploitation of whey as a source of other products has not been carried out on a large scale except under wartime emergency conditions. The cost of treating whey as a waste is increasing; in some parts of the United States the cheesemaker pays up to 3 cents/cwt. to have the whey hauled from the plant and in many communities the installation of waste treatment plants is required by law. It would be desirable to process the whey so that the cheesemaker could realise a profit on the product; but even if the returns only just balanced the cost, the expense of treating whey as a waste would still be saved.

Advantages of yeast protein

Whey can be converted into animal protein when used as slops for farm animals. In many countries the cheese plant may have an adjacent pig farm as an outlet for the whey. However, the amount of whey consumed per animal is limited, and changing whey into animal protein is an uneconomical process. Approximately 15 lb. of whey protein is required to make 1 lb. of animal protein[2]. The approximately 1,700 lb. of whey necessary to supply this protein contains enough sugar to produce 18 to 20 lb. of yeast protein. Yeast protein can be obtained in a matter of hours, while months are needed to produce animal protein. However, the processes reported in the literature for growing yeast on whey have not proved satisfactory, and the cost of this yeast protein has not been competitive with other yeast and plant proteins. Re-evaluation of yeast propagation methods has led to the development of a process that may make the production of yeast from whey commercially feasible.

The important modifications in the propagation process are as follows:

1. The supply of the proper quantities of medium supplements.
2. The size of the yeast inoculum to reduce propagation time.
3. The use of raw whey and non-sterile operating techniques to reduce the cost of equipment and labour.
4. The supply of a quantity of oxygen sufficient for the maximum rate of growth of the yeast.

*Eastern Regional Research Laboratory, Eastern Utilisation Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

Aaron E. Wasserman is in charge of the whey utilisation investigations of the dairy products laboratory, U.S. Department of Agriculture. He took his B.S. degree in bacteriology at the Philadelphia College of Pharmacy and Sciences and carried out his graduate studies in bacteriology and biochemistry there and at the Massachusetts Institute of Technology. He has been on the staff of the Eastern Utilisation Research and Development Division since 1954 and before that was employed in the pharmaceutical industry investigating problems in bacterial metabolism and drug action.

Laboratory studies

Lactose is the chief source of available carbohydrate in whey. Growth of an organism will depend on its ability to utilise this sugar, and the species of yeast capable of assimilating lactose are limited. *Torula cremoris* was considered a satisfactory lactose fermenter by Rogosa[4] and Graham *et al*[5]. *Torula utilis*, which grows well in molasses and wood sulphite waste liquor, was grown in whey by the Waldhof process[6], although other workers indicate that this yeast cannot utilise lactose[5]. *Saccharomyces fragilis* grew more rapidly in whey than several other species of yeasts[7], and on the basis of the preliminary findings of Porges *et. al*[8]. in this laboratory, the NRRL strain of *S. fragilis* Y1109 was used for the rapid conversion of whey substance to yeast protein. Other strains of yeast, although not investigated, may be equal to *S. fragilis* under suitable growing conditions.

The medium

The lactose composition of whey varies between 4 and 5%, depending on the variety of cheese produced. Satisfactory yeast yields have been obtained from cottage or Cheddar cheese whey. The whey from Ricotta or Italian curd cheeses, which are made with quantities of whole milk, is also a satisfactory medium for yeast growth, but the higher fat content may cause difficulties in drying the yeast product.

Although whey contains nitrogen, phosphorus and other salts, supplementation is necessary for maximum yeast yields. Phosphorus, in 0.225% concentration, can be added satisfactorily as the mono- or di-salt of either sodium or potassium. The nitrogen content of whey at 0.13% is insufficient to produce the maximum yield of cells containing the 40 to 50% protein required for food or feed yeast. Only about 25% of the whey nitrogen is actually available to the yeast, and this nitrogen is in the organic fraction soluble after heat- and acid-precipitation of the whey proteins[9]. The addition of N, as $(NH_4)_2SO_4$, results in increased yeast yields, and the N content of the yeast depends on the quantity of $(NH_4)_2SO_4$ added. Table 1 indicates that the addition of 0.5% $(NH_4)_2SO_4$ results in an excellent yield of yeast containing 10% less N than the original seed. The addition of 1% $(NH_4)_2SO_4$ produces a crop of cells of equivalent weight, but having 12% more N than the seed. At 1% $(NH_4)_2SO_4$, unused inorganic N remains in the discard liquor. As this would be wasteful, a quantity between 0.5 and 1% $(NH_4)_2SO_4$ should be used. The ratio of carbon to available N in whey is approximately 48 : 1. In yeast the average carbon to N ratio is 7 : 1. The whey, therefore, is deficient in N to support growth of yeast of the required C : N ratio. The addition of approximately 0.85% $(NH_4)_2SO_4$ is sufficient to correct the deficiency in whey with no excess N remaining in the supernatant.

Table I—Effect of Nitrogen Supplementation of Whey on the Yield and Nitrogen Contents of *S. fragilis*

	Whey alone	Whey + $(NH_4)_2SO_4$ 0.5%	Whey + $(NH_4)_2SO_4$ 1.0%
Net yield, gm. dry wt./L*	14.6	24.12	23.04
% Cell N**	4.93	6.62	8.17

* Initial inoculum was 13 gm. dry wt./L.
** Initial cell N was 7.3%.

S. fragilis requires a growth factor(s) present in yeast extract or dried brewers' yeast[10]. In the absence of this supplement, yeast yields decline. The factor can apparently be stored within the growing yeast, since yeast originally grown in fortified media and then transferred to media without yeast extract, only gradually decreased in yield. The required growth factor has not been identified, but it does not appear to be among the usual vitamins or growth-promoting substances. No investigations have been made for other plant and animal preparations that may be substituted for yeast extract.

The optimum growth of *S. fragilis* occurs at pH 5.0 to 5.7[10]. Under conditions of active growth, the pH tends to maintain itself within these

yeast yield might be expected by using a more efficient oxygen dissolving system, and by converting from batch to semi-continuous or continuous operation.

To produce 100 lb. dry yeast from cheddar cheese whey, the following components are required:

Lactose	175 lb. (in 3,500 lb. whey)
$(NH_4)_2SO_4$	35 lb.
KH_2PO_4	18 lb.
Yeast extract	4.2 lb.
Oxygen (approx.)	88 lb.

Nutritional value of S. fragilis.

The need for protein in human and animal nutrition is a well recognised fact. Yeast have traditionally been considered an excellent source of such protein. Strains of *Saccharomyces cereviseae* and *Torulopsis*, primarily, have been used for dietary purposes. Torula yeast is usually grown specifically for protein on molasses. Within recent years, however, commercial production of torula yeast has been carried out in sulphite liquor, a waste product of the paper pulp industry.

Many reports have appeared on the amino acid and vitamin composition of various yeast. Although variations in the reported values occur, due perhaps to difference in experimental technique, yeast strain, or yeast nutrition, the concentrations of the different amino acids and vitamins are comparable. However, among all the data in the literature examined, no analyses for *S. fragilis* could be found. It was expected that *S. fragilis* would conform to the compositional pattern observed with other yeast strains, but analyses were carried out to confirm this. Amino acids were determined by the Moore and Stein method[17] and vitamins by conventional microbiological or chemical procedures. The data are shown in Table 3.

Preliminary results indicate that the amino acid composition of *S. fragilis* is similar to the average pattern for 17 yeasts described by Block and Bolling[18]. Lysine, however is present in a somewhat greater concentration, a fact that may be of interest in view of the importance of lysine in the present concept of amino acid requirements.

The vitamin composition of *S. fragilis* also appears to be similar to that for other yeasts, except for a low thiamin content. Further work should show whether the low vitamin B_1 concentration is a characteristic of this strain of yeast or whether it is the result of some experimental procedure (*i.e.* an imbalance in the growth medium constituents, loss during the washing of the yeast cells, or destruction during drying).

Table 3 — The Amino Acid and Vitamin Composition of *S. fragilis*

Amino Acids (gm/16 gm N)			
Lysine	10.20	Glycine	4.63
Arginine	7.08	Alanine	8.17
Histidine	1.87	Valine	7.78
Asparagine	11.16	Methionine	1.25
Threonine	6.46	Isoleucine	6.00
Serine	6.96	Leucine	9.60
Glutamic Acid	13.26	Tyrosine	3.42
Proline	4.31	Phenylaline	5.39

Vitamins			
Choline	6.67 mg/gm	Niacin	280 μ/gm
Inositol	3.0 mg/gm	Folic acid	6.83 μ/gm
Thiamin	24.1 μ/gm	Pantothenic acid	67.2 μ/gm
B_6	13.6 μ/gm	p-amino benzoic acid	24.2 μ/gm
Riboflavin	36.0 μ/gm	Biotin	1.96 μ/gm

The yeast was compared with casein as the sole source of protein in a weight-gain test with young rats. *S. fragilis* was used by the rats as efficiently as *T. utilis*[19]. There is a difference, however, in the weight gains induced by *S. fragilis* and casein, indicating a deficiency of some amino acid in the *S. fragilis* protein. Yeast are low in the sulphur amino acids (methionine and cystine) and supplementation of the animal diet with these amino acids increased the biological value in feeding tests[19]. Further tests with *S. fragilis* probably will also show that the addition of sulphur amino acids is necessary to raise the value of the protein.

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Oxygen utilisation

Oxygen consumption by *S. fragilis* during the dissimilation of lactose was determined initially in a Warburg manometric apparatus[10]. While the yeast preferentially used glucose when both glucose and galactose (the components of lactose) were in the medium, the course of lactose utilisation indicated that the sugar is taken into the cell without prior hydrolysis. The yeast utilise only about 35% of the total amount of oxygen necessary to oxidise a given quantity of lactose to CO_2 and water. Approximately the same quantity of CO_2 is formed. The remaining 65% of the lactose carbon is used for new cell material or non-oxidisable metabolic products. Thus, knowing the quantity of lactose present in the whey, the total amount of oxygen necessary for the utilisation of the sugar and maximum growth may be calculated. This was confirmed in the laboratory propagators, where the oxygen utilisation of the growing yeast was determined by measuring the oxygen content of the effluent air with an oxygen analyser. In media prepared with cottage cheese whey containing 4% lactose, the 40 gm. of sugar should require 10.5 l. (or 14.5 gm.) of oxygen during the growth of the yeast. This is the minimum amount of oxygen the yeast will need, regardless of the time required to complete the propagation.

Observations during the course of several hundred propagations made with 500 ml. and 15 l. volumes of medium showed that between 13 and 15 l. of oxygen per litre of suspension were consistently utilised by the yeast. The oxygen consumed in excess of the anticipated quantity (10.5 l.) is undoubtedly due to the endogenous respiration of the yeast, which will vary depending on the age and physiological condition of the culture. Some oxygen will also be consumed in oxidising the small quantities of protein and lactic acid present in the whey.

Table 2 — Yield Data of a Representative 4 hr. Pilot Plant Propagation of *S. fragilis*

Volume whey	600 gal.
Lactose present (4.86%)	242 lb.
Lactose used	242 lb.
Volume seed yeast	150 gal.
Weight of seed yeast	110 lb.
Final vol. of suspension	750 gal.
Gross dry wt. of yeast	215 lb.
Net dry wt. of yeast	105 lb.
Theoretical yield (55% of utilised lactose)	133 lb.
% Theoretical yield	79%

Oxygen requirements

The O_2 demand of growing yeast increases to a peak and then declines rapidly, as shown in Fig. 2[13]. Therefore it is not sufficient to know the total oxygen requirement of the yeast. If the total oxygen demand is supplied in equal increments, there will be excess aeration at certain periods of the growth phase and insufficient aeration at other times. Anaerobic conditions occur quickly in the absence of sufficient oxygen, and limit the yeast growth. The peak oxygen requirements for the growth of *S. fragilis* carried out under the conditions described are in the order of 100 to 120 ml. O_2/l. medium/min. (3.5 to 4.0 ml. O_2/mg. dry wt. yeast/min.). This is the minimum quantity of oxygen that must be supplied for maximum yeast yield.

The quantity of oxygen dissolved into the propagation medium depends on the efficiency of the aeration and agitation equipment. An approximation of the oxygen absorption rate of a propagator can be obtained by the sulphite oxidation method of Cooper *et. al.*[14]. However, the rate of oxygen solution in a medium may differ from the rate of solution in sodium sulphite, and the growth of the yeast may be unexpectedly affected. Good yields of yeast were obtained in a Waldhof propagator under conditions of aeration and agitation that dissolved, in sodium sulphite solution, only about 60% of the oxygen required by the yeast at peak demand[15].

Pilot plant studies

Yeast was grown in 1,000 gal. quantities of whey medium in a 1,600 gal. vat[16]. The aeration-agitation equipment was designed to transfer at least 425 ml. oxygen/gal./min. to satisfy the peak O_2 demand of the yeast. When tested with the sulphite oxidation method, however, considerably less oxygen was actually transferred. Finally, after installing baffles, changing the design of the agitator and reducing the liquid volume to 800 gal., an oxygen transfer rate of 310 ml./gal./min. was reached. The propagation of the yeast was begun, with the realisation that reduced yields could be expected.

Results obtained in a representative 4 hr. yeast propagation are shown in Table 2. Removal of lactose from the medium was essentially complete in 4 hr. and the maximum yeast growth under these conditions was attained. Increased

limits. At times, the pH may approach 6.0 and, if not corrected, will become alkaline, reaching values as high as pH 8.5. Yeast growth is greatly reduced under these conditions. The pH may be maintained below pH 6.0, either by the addition of acid, or by shutting off the air supply to the propagator for 5 to 10 min., allowing the yeast to form their own acid under the anaerobic conditions that are quickly established.

Although the yeast is propagated in the laboratory at 31 to 33°C., good yields were obtained at temperatures as high as 41 to 43°C. At the higher temperatures, however, contamination with a rod-shaped organism occurred. Since large quantities of heat are produced during yeast growth, the wide range of temperatures within which the yeast grow well is important in considering the cooling requirements of plant-size propagations.

Yeast inoculum

The yeast yield is limited by many factors, but under a given set of conditions the time required to reach the yield limit is dependent on the size of inoculum, as shown in Fig. 1[11]. With a small seed (3.5 gm./l.) the maximum yield is attained in 8 hr., but with an inoculum of 23.8 gm./l. the maximum is reached in 3 to 4 hr. Although the yield is more impressive (nine-fold increase) with the smaller yeast inoculum than with the larger seed (two-fold increase), the actual formation of new cell material is the same (23 gm./l.). Seeding with yeast equal in dry weight to approximately 30% of the weight of the lactose in the medium results in a 4 hr. growth cycle.

Non-sterile conditions

The literature reports that heat precipitation of whey proteins results in improved yeast yields. Whey, heat-treated at pH 1.5 to 3.5, gave maximum yeast yield when adjusted to pH 7.0 for propagation[12]. Thus, the heat treatment, in addition to sterilising the whey, apparently altered the medium to make it more favourable for yeast growth. It is possible that the lactose was hydrolysed under these conditions, liberating some glucose which is more readily utilised by the yeast. This may explain the stimulatory effect of acid- and heat-deproteinisation.

The heat treatment of whey on a large scale entails an increased outlay for equipment—heat exchangers, storage tanks, and centrifuges or filters to remove the precipitated protein. Propagation of yeast under sterile conditions involves complicated operations for sterilising, and maintaining sterility in, storage tanks, propagator vats, air and water supply, and all other equipment used in growing the yeast.

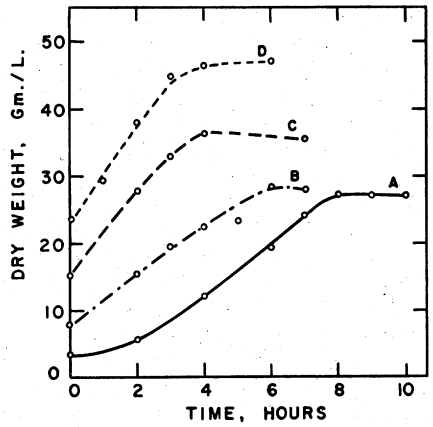

Fig. 1. The effect of inoculum size on the growth rate and yield of S. fragilis in whey medium. Initial inoculum sizes were A—3.5 gm./l., B—7.9 gm./l., C—15.1 gm./l. and D—23.8 gm./l.

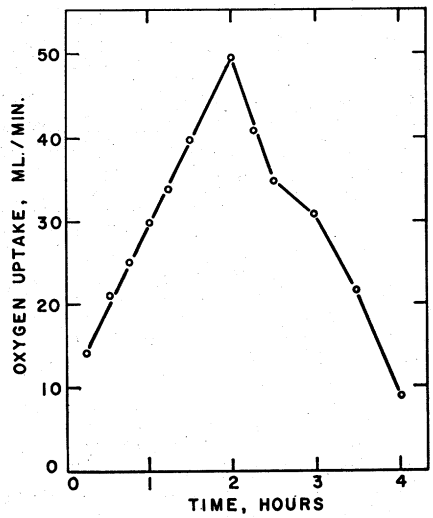

Fig. 2. The oxygen consumption of S. fragilis growing in 500 ml. whey medium and aerated at 2 l./min.

However, it has been shown that, with the rapid growth rate of the yeast and the short duration of a cycle (4 hr.), the yeast will outgrow contaminating organisms. Experiments have been carried on during a period of years in which the inocula were successively transferred from previous yeast propagations and contamination was a problem only at higher growth temperatures. Yeast grow as well in a raw whey medium as in medium prepared from heat-treated, deproteinised whey.

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